

Insuetifurca austronipponica, a New Tardigrade (Eutardigrada: Macrobiotidae) from Kagoshima, Southern Japan

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ABSTRACT—A new semiterrestrial tardigrade, *Insuetifurca austronipponica*, is described from Kakeroma Island, Kagoshima Prefecture, southern Japan. This is the third species known for the genus. It is distinguished from *I. fujiensis* primarily by a narrower buccopharyngeal tube and a longer pharyngeal tube, and from *I. arrowsmithi* primarily by a microplacoid clearly shorter than the macroplacoids.

Key words: taxonomy, water bear, Tardigrada, eutardigrade, *Pseudodiphascon*

INTRODUCTION

Species of Macrobiotidae with a flexible pharyngeal tube have long been assigned to *Pseudodiphascon*, which was erected by Ramazzotti (1965) as a subgenus of *Macrobiotus* Schultze, 1834, and later raised to generic status by Pilato (1969a, b). However, Ito (1997) briefly but explicitly expressed doubts about the taxonomic unity of species within *Pseudodiphascon*. More recently, Guidetti and Pilato (2003) published an extensive taxonomic revision based mainly on the reexamination of type specimens. They documented remarkable heterogeneity in the morphologies of the buccopharyngeal apparatus and claw among species of *Pseudodiphascon*, split this genus, and established three new genera: *Biserovus*, *Insuetifurca*, and *Minilentus*. They also showed that the type species of *Pseudodiphascon*, *Macrobiotus inflexus* Arcidiacono, 1964, is a dubious species described from specimens in the molting simplex stage, and thus they considered *Pseudodiphascon* also to be a dubious name. As a result of the revision by Guidetti and Pilato (2003), *Insuetifurca* included two species: *I. arrowsmithi* (Kathman and Nelson, 1989) from Vancouver Island, Pacific Canada and *I. fujiensis* (Ito, 1997) from Mt. Fuji, central Japan.

Examination of the tardigrades extracted from a foliose lichen from Kakeroma Island, Kagoshima Prefecture, southern Japan resulted in the discovery of a previously unreported species of *Insuetifurca*, which is described here as the third species of the genus.

MATERIALS AND METHODS

A foliose lichen was scraped off the substrate, stored in a paper envelope to prevent mold, and brought back to the laboratory. The lichen sample was stirred gently and soaked overnight in tap water in a petri dish. Living tardigrades released onto the bottom of the petri dish were picked up under a dissecting microscope at 30× magnification, killed by heating, and prepared for slides by the method described in Abe and Takeda (2000). Hoyer's medium was used as a mountant, and cover slips were sealed with Murrayite.

The specimens were examined by phase contrast and Nomarski differential-interference microscopy at the highest magnification (×1250), measured with a calibrated ocular micrometer, illustrated with the aid of a camera lucida, and photographed with a digital camera attached to the microscope. Buccopharyngeal tube length was measured from the posterior dorsal crest in the buccal cavity to the proximal base of the buccopharyngeal tube, excluding the apophysis in the pharyngeal bulb; *pt* and *pbf* values were calculated as the percentage ratios between the measurement of any given structure and the lengths of the buccal tube and buccopharyngeal tube, respectively (Pilato, 1981; Pilato *et al.*, 2002). Morphological terminology primarily follows the usage of Bertolani and Rebecchi (1993).

The type specimens of the new species are deposited in the collection of the Department of Zoology, National Science Museum, Tokyo (NSMT).

TAXONOMIC ACCOUNT

Insuetifurca austronipponica sp. nov.

(Figs. 1, 2; Table 1)

Diagnosis

Teeth developed in anterior part of buccal cavity; buccopharyngeal tube narrow; pharyngeal bulb elongate-oval, with 3 macroplacoids and 1 microplacoid; macroplacoid 1 the longest; macroplacoid 2 slightly shorter than macroplacoid 3; microplacoid obviously shorter than macroplacoids;

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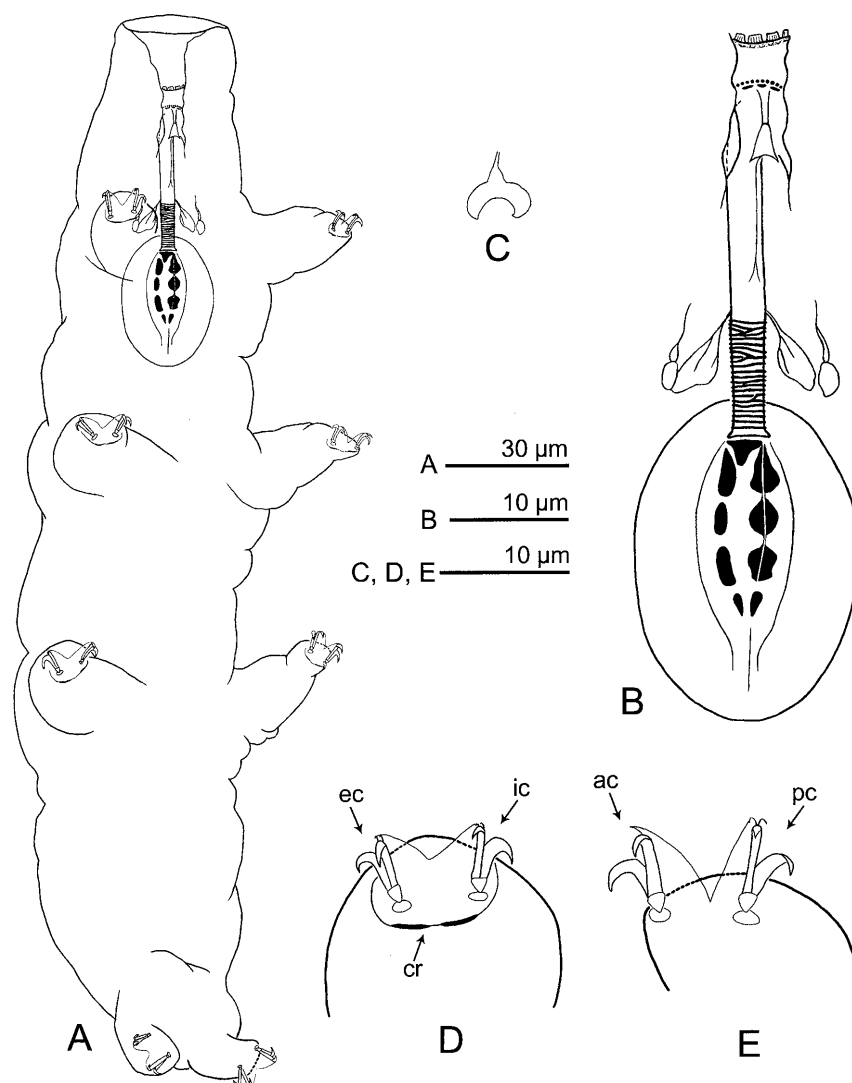


Fig. 1. *Insuetifurca austronipponica* sp. nov., A, B, D, E, holotype; C, paratype (NSMT-Tg113). (A) Habitus, ventral view. (B) Buccopharyngeal apparatus, ventral view. (C) Furca, lateral view. (D) Leg 3, ventral view. (E) Leg 4, ventral view. ac, anterior claw; cr, cuticular ring; ec, external claw; ic, internal claw; pc, posterior claw.

claws with small, smooth lunules.

Etymology

The specific name "*austronipponica*" is a feminine adjective in the nominative singular; *austro* (Latin) means southern and *nipponica* (Latinized Japanese) means Japan, referring to the type locality of the new species (southern Japan).

Type series

Holotype: animal of undetermined sex (NSMT-Tg 111); Nishi-amuro, Setouchi, Kakeroma Island, Amami Islands, Kagoshima Prefecture, Kyushu, southern Japan; 28°06'41"N, 129°13'17"E; 4 m elevation; extracted from a foliose lichen growing on rock; 22 January 1997; collected by M. Mitsuhashi. Paratypes: two animals of undetermined sex (NSMT-Tg 112 and 113); other collection information as for holotype.

Description of holotype

Body elongate, 238.4 μ m long excluding leg 4, 51.8 μ m wide (Figs. 1A, 2A); color in life unknown; evenly translucent in preserved material. Body surface smooth, without pores or granulation. Eyespots absent.

Buccopharyngeal apparatus 61.6 μ m long from mouth opening to posterior end of pharyngeal bulb, 25.8% of body length (Figs. 1A, B, 2A, B). Mouth situated subterminally, 4.6 μ m in diameter, surrounded with 10 peribuccal lamellae. Buccal armature subcylindrical, its anterior-most part armed with several small transverse teeth; faint, irregular decoration developed around teeth; medial portion of buccal armature smooth, without sculpture; posterior band developed just above dorsal crests near posterior edge of buccal armature, composed of miniscule teeth which are transversely positioned and spaced closely to one another; each tooth rounded on the dorsal side and slightly elongated on the

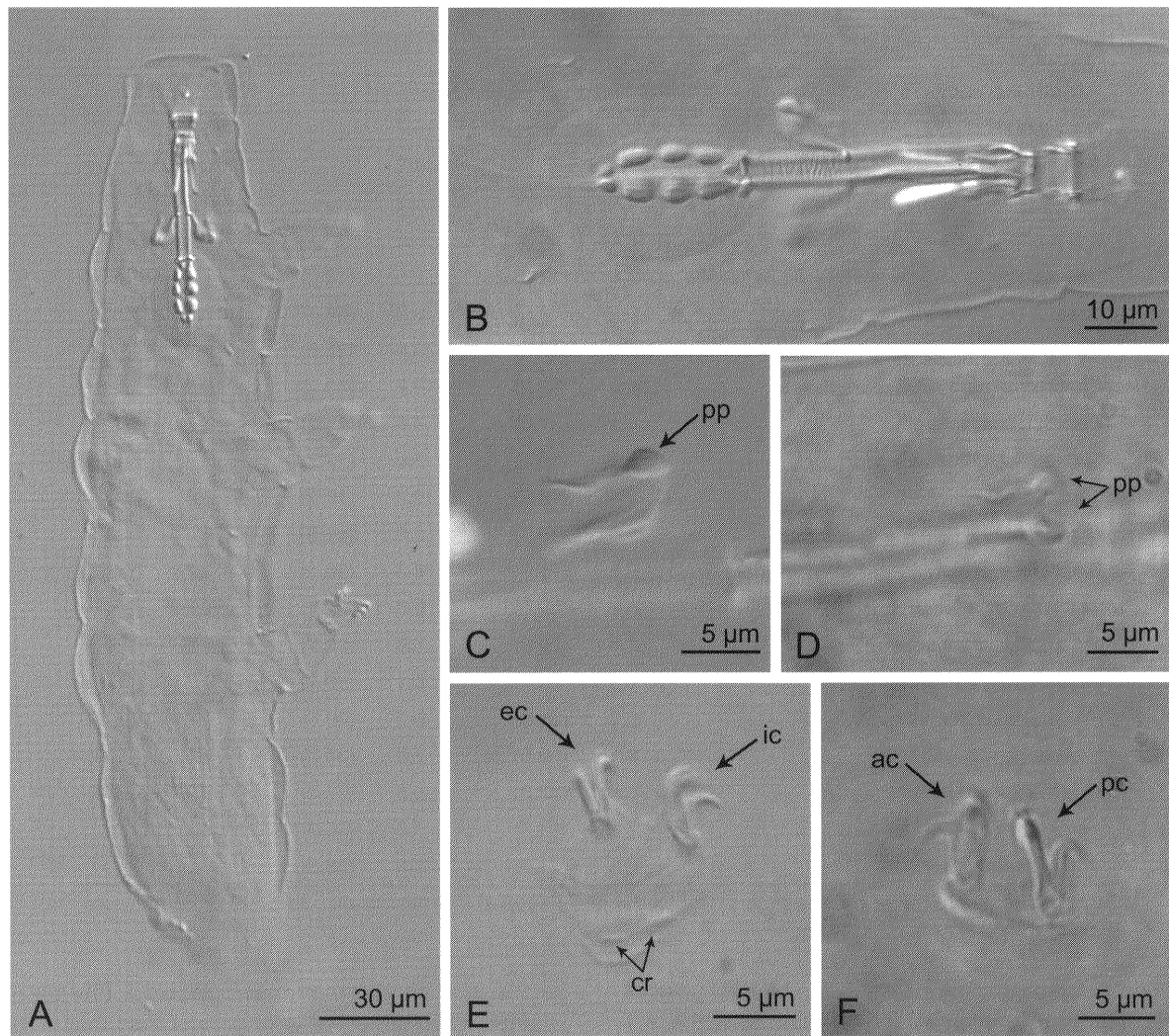


Fig. 2. *Insuetifurca austronipponica* sp. nov., differential interference micrographs. A–C, holotype; D, paratype (NSMT-Tg 113); E, F, paratype (NSMT-Tg 112). (A) Habitus, ventral view. (B) Buccopharyngeal apparatus, ventral view. (C) Furca, ventral view. (D) Furca, lateral view. (E) Leg 3, ventral view. (F) Leg 4, ventral view. ac, anterior claw; cr, part of the cuticular ring on leg; ec, external claw; ic, internal claw; pc, posterior claw; pp, posterior process of furca.

ventral. Dorsal crests consisting of 3 transverse bars; ventral crests positioned beneath posterior band, consisting of 2 lateral and 1 medial transverse bars; medial bar slightly shorter than lateral bars. Buccopharyngeal tube narrow, length 32.5 μm and internal diameter 2.5 μm (*pt* value of buccopharyngeal tube width 12.3), slightly curved, divided into buccal tube (rigid anterior portion) and pharyngeal tube (flexible posterior portion) (Figs. 1B, 2B); buccal tube provided with strengthening bar ventrally; boundary between buccal and pharyngeal tubes situated below level of insertion of stylet support; outer wall of pharyngeal tube strengthened with cuticular thickening; cuticular thickening subspiral, but not complete, *i.e.*, adjacent spirals merged or ramified at some points (Figs. 1B, 2B). Furca typical in size for the genus, with two well developed processes that are arched and converge posteriorly (Figs. 1C, 2C, D).

Pharyngeal bulb elongate-oval, 29.3 μm long and 18.9 μm wide (Figs. 1B, 2B). Apophysis in pharyngeal bulb

rounded, inverted-subtriangular. All macroplacoids short, rod-shaped in dorsal view, well demarcated from one another (Figs. 1B, 2B); macroplacoid 1 the longest, clearly longer than macroplacoids 2 and 3; macroplacoid 2 slightly shorter than macroplacoid 3; macroplacoids 1, 2, and 3 are 4.2, 3.3, and 3.4 μm long, respectively; macroplacoid 3 with blunt and very shallow constriction posterodorsally. Microplacoid tapering sharply posteriorly, 2.0 μm long, distinctly shorter and smaller than macroplacoids, located near macroplacoid 3; total placoid row length 15.5 μm (*pt* value 76.8).

All claws similar to one another in size and shape (Figs. 1D, E, 2E, F). Each claw provided with a small but evident oval lunule at the base; edge of lunule smooth, without dentation; common tract of each claw small, an inverted triangle, attached with an extremely short stalk inserted at center of lunule. Primary and secondary branches ramified basally, just above common tract; configuration of claw thus Y-shaped. Primary branches of all claws provided with 2 con-

Table 1. Morphometric data of *Insuetifurca austronipponica* sp. nov.

Registration numbers	Holotype		Paratypes	
	NSMT-Tg 111	NSMT-Tg 112	NSMT-Tg 113	
Body length	238.4	215.7	170.7	
Body width	51.8	44.2	50.4	
Mouth opening diameter	4.6	5.2	3.3	
Buccopharyngeal tube length	32.5	30.6	27.3	
Buccopharyngeal tube internal diameter	2.5 (12.3)	2.5 (12.2)	2.2 (11.8)	
Stylet support insertion level	20.2 (62.0)	20.3 (66.4)	18.2 (66.7)	
Pharyngeal bulb length	29.3 (145.2)	25.4 (125.0)	22.8 (125.3)	
Pharyngeal bulb width	18.9 (93.5)	18.2 (89.7)	16.9 (92.9)	
Macroplacoid 1 length	4.2 (21.0)	3.9 (19.2)	3.3 (17.9)	
Macroplacoid 2 length	3.3 (16.1)	3.6 (17.7)	2.3 (12.9)	
Macroplacoid 3 length	3.4 (16.8)	3.7 (18.2)	2.6 (14.3)	
Microplacoid length	2.0 (9.7)	2.6 (12.8)	1.6 (9.0)	
Placoid row length	15.5 (76.8)	16.3 (80.1)	11.7 (64.3)	
Internal claw 1 length	6.1 (30.0)	6.6 (32.7)	4.7 (25.7)	
External claw 1 length	5.9 (29.0)	6.6 (32.7)	4.6 (25.0)	
Internal claw 2 length	5.9 (29.0)	6.1 (30.1)	5.2 (28.6)	
External claw 2 length	6.3 (31.3)	6.2 (30.5)	5.2 (28.6)	
Internal claw 3 length	6.1 (30.0)	7.0 (34.3)	5.1 (27.9)	
External claw 3 length	6.5 (32.3)	5.5 (27.1)	5.2 (28.6)	
Anterior claw 4 length	7.8 (38.7)	7.2 (35.3)	–	
Posterior claw 4 length	8.3 (41.2)	7.8 (38.5)	–	

Measurements are given in micrometers. *Pbf* (of the stylet support insertion level) and *pt* (of other measurements) values are shown in parentheses.

spicuous and sharp accessory points at the highest point of the branch, near the tip. Internal claws 1–3 are 6.1, 5.9, 6.1 μm long, and external claws 1–3 are 5.9, 6.3, 6.5 μm long, respectively; anterior and posterior claws 4 are 7.8 and 8.3 μm long, respectively.

Measurements and *pt* and *pbf* values of each taxonomic character are listed in Table 1.

Notes on paratypes

Body surface smooth. Eyespots absent. Buccal armature provided with anterior teeth. Buccopharyngeal tube narrow (*pt* values of buccopharyngeal tube internal diameter 11.8 and 12.2). *Pbf* values for the insertion of the stylet supports on the buccopharyngeal tube 66.4 and 66.7. Pharyngeal bulb elongate-oval. Macroplacoid 1 the longest; macroplacoid 3 slightly longer than macroplacoid 2. Microplacoid clearly shorter and smaller than macroplacoids.

Measurements and *pt* and *pbf* values of each taxonomic character are listed in Table 1.

Differential diagnoses

Insuetifurca austronipponica sp. nov. is the third species for the genus and is readily distinguished from *I. arrowsmithi*, known only from its type locality, Vancouver Island, Pacific Canada, in having 1) a microplacoid distinctly

smaller and shorter than macroplacoid 2 (the microplacoid is large, its length equal to or greater than that of macroplacoid 2 in *I. arrowsmithi*) and 2) an elongate-oval pharyngeal bulb (the pharyngeal bulb is spherical in *I. arrowsmithi*). The new species differs from the other species, *I. fujiensis*, known only from Mt. Fuji, Yamanashi Prefecture, Central Japan, in having 1) a clearly narrower buccopharyngeal tube (*pt* value of buccopharyngeal tube width is 11.8–12.2) relative to its length (*pt* value of buccopharyngeal tube width ca. 20.4 in holotype of *I. fujiensis*, measured from the illustration in Ito [1997]) and 2) a longer pharyngeal tube. Guidetti and Pilato (2003) also illustrated the buccopharyngeal apparatus of *I. fujiensis*, and it is clear that *I. fujiensis* has a wider buccal tube and a shorter pharyngeal tube.

DISCUSSION

Insuetifurca is one of the three genera recently split from *Pseudodiphascon* by Guidetti and Pilato (2003). The new species reported here should be included in *Insuetifurca* because of the following characters: 1) the pharyngeal tube is flexible, with spiral thickening, 2) the claws are Y-shaped in configuration and with lunules basally, and 3) peribuccal lamellae are present in the anterior part of the buccal armature.

Guidetti and Pilato (2003) pointed out that *Insuetifurca arrowsmithi*, the type species of the genus, has a peculiar furca with posterior processes arched and converging backwards. I concur that this peculiarity in the furca is an important character justifying separation of *Insuetifurca* from other genera of Macrobiotidae (especially from *Biserovus* and *Minilentus*). A similar and peculiar furca was observed also in *I. austronipponica* sp. nov.

In Macrobiotidae, cuticular rings on legs 1–3 have been observed only in *Calcaribiotus* (Dastych, 1993, as cuticular bars; Abe and Takeda, 2000), and Dastych (1993) considered this to be one of the taxonomic characters that discriminates *Calcaribiotus* from the other genera in the family. I clearly observed cuticular rings on legs 1–3 in *Insuetifurca austronipponica* (Figs. 1D, 2E). Nevertheless, this does not necessarily imply a close relationship of the two genera within Macrobiotidae, because this character commonly occurs in some species of other genera of Macrobiotidae (Abe, unpublished data). It is usually difficult to confirm the presence of this character, especially when the specimen is not fully stretched or is not fixed in an adequate position.

Most genera of semiterrestrial eutardigrades have a cosmopolitan distribution (McInnes, 1994), including in Europe, which is the best-studied area for tardigrade taxonomy and biogeography. Species of *Insuetifurca* have, however, not been reported from Europe, and this suggests that the taxon is absent from the region. Although it is necessary to investigate additional localities, *Insuetifurca* appears to have a trans-Pacific distribution in the northern hemisphere, based on one locality from Pacific Canada and two localities from Japan. Such a distributional pattern is previously unknown in tardigrade biogeography.

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